Hystrix. (n.s.) 6 (1-2) (1994): 77 - 97 (1995) Proc. II Conf. on Dormice

BIOCHEMICAL SYSTEMATICS AND EVOLUTION OF MYOXIDAE

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ABSTRACT – Genetic variation and divergence were analysed among 43 populations representing the five western Palaearctic genera of the family Myoxidae: *Myoxus, Myomimus, Muscardinus, Eliomys* and *Dryomys.* Intraspecific and interspecific genetic divergence were evaluated by electrophoretic analysis of 38-42 gene loci and compared with data from fossil records. The mean values of heterozygosity per locus for each species ranged from 0.024 in *Myomimus roachi* to 0.062 in *Dtyomys nitedula.* The mean values of intraspecific genetic distance ranged from 0.04 in *Dryomys nitedula* to 0.081 in *Eliomys melanurus*. **A** comparison of the five genera, based on 38 gene loci indicated a high level of differentiation. Only two loci were found monomorphic and fixed for the same allele in the five genera: *Ldh-2* and *Got-2*. The lowest mean value of genetic distance was observed between the genera *Myoxus* and *Eliomys* (D = 1.283). *Muscardinus* displayed a higher value of genetic distance in comparison with *Myoxus* and *Eliomys* (D = 1.629). The highest mean value of genetic distance was displayed by these three genera in comparison with *Myomimus* and *Dryomys* (D= 2.251). A high value of genetic distance was also observed between *Myomimus* and *Dryomys:* D = 1.811. These values are in agreement with the ancient origin of this family, that had its highest expansion and diversification during the Miocene.

Key words: Myoxidae, Allozyme variation, Biochemical evolution.

RIASSUNTO – *Sistematica biochimica ed evoluzione dei Myoxidae* – Sono stati analizzati variabilita genetica e differenziamento genetico in 43 popolazioni rappresentanti i cinque generi paleartico-occidentali della famiglia Myoxidae: *Myoxus, Myomimus, Muscardinus, Eliomys* e *Dryomys*. La divergenza genetica intra- ed interspecifica è stata valutata mediante analisi elettroforetica di 38-42 loci genici e confrontata con dati derivati da reperti fossili. I valori medi di eterozigosi per locus per ogni specie sono compresi tra 0,024 in *Myomimus roachi* e 0,062 in *Dryomys nitedula.* I valori medi di distanza genetica intraspecifica sono compresi tra 0,04 in *Dryomys nitedula* e 0,081 in *Eliomys quercinus*. Un confronto fra i cinque generi, basato su **38** loci genici, ha indicato un elevato livello di differenziamento. Soltanto due loci sono risultati monomorfici e fissati per lo stesso allele nei cinque generi: *Ldh-2* e *Got-2*. Il piu basso valore medio di distanza genetica è stato osservato tra i generi *Myoxus* e *Eliomys* (D = 1,283). *Muscardinus* presenta un valore di distanza genetica maggiore in confronto con *Myoxus* ed *Eliomys* (D = 1,629). Il valore piu elevato di distanza genetica e stato osservato confrontando questi tre generi con *Myomirnus* e *Dryomys* (D = 2,251). Un elevato valore di distanza genetica e stato anche osservato tra *Myomimus* e *Dryomys* (D = 1,811). Questi valori sono in accordo con I'antica origine di questa famiglia, che ha avuto la massima espansione e diversificazione durante il Miocene.

Parole chiave: Myoxidae, Variabilita genetica, Evoluzione biochimica.

INTRODUCTION

The understanding of microevolutionary processes leading to speciation has been improved in the last twenty years by the application of electrophoretic techniques to population genetics and by the introduction of chromosomal banding techniques to cytogenetic analysis.

In many taxa of small mammals, particularly rodents, the mechanisms of speciation often involve chromosomal rearrangements. Therefore karyotype analysis assumes a significant value and the chromosomes involved in such rearrangements can be identified by banding techniques. Among Myoxidae, a process of speciation through karyotypic differentiation is present in the genus *Eliomys*. Previously, four distinct karyotypes, originated through successive Robertsonian fissions and characterized by parapatric distribution, were found in *Eliomys quercinus* (2n = 48, 50, 52, and 54; see Filippucci et al., 1988 a, 1990) in Europe and two karyotypes in *Eliomys melanurus* from North Africa (2n = 46) and from lsrael (2n = 48). Details of karyotype variation are given in Filippucci et al. (1988 a and b, 1990). Phylogenetic relationships among the chromosomal forms of *Eliomys* were clarified by electrophoretic analysis of gene-enzyme systems (Filippucci et al., 1988 c).

The use of electrophoretic techniques allows evaluation, by means of appropriate statistical methods, of the amount of genetic variation and divergence among populations, geographical races, species and genera. Through electrophoresis it is possible to estimate time of divergence, to clarify taxonomic status and phylogenetic relationships between taxa, and to evaluate the evolutionary significance and timing of karyotype differentiation. This technique can also reveal the existence of sibling species that are undetectable at the morphological level.

Information about the genetic structure of populations in dormouse species, gained through electrophoretic analysis of gene-enzyme systems, is completely lacking except for *Eliomys* (Filippucci et al., 1988 c).

In the present study genetic variation and divergence were analysed among 43 populations representing the five western Palaearctic genera of the family Myoxidae: *Myomimus*, *Myoxus, Dryomys*, *Muscardinus*, and *Eliomys*. The electrophoretic analysis was carried out on 38-42 gene loci. The genetic differentiation is evaluated and discussed at several stages of divergence (among populations, subspecies, species and genera) and compared with paleontological evidence.

MATERIAL AND METHODS

298 specimens belonging to the genera ***Myoxus,*** *Myomimus*, *Muscardinus, Eliomys* and *Dryomys* were analysed for electrophoretic variations. In the present study are included populations of the genera *Eliomys* and *Dryomys* previously analysed (Filippucci et al., 1988 c; Vujošević et al., 1993; Filippucci et al., 1995).

The number of specimens examined, their collecting sites, and sample designations are given in Table 1. In *Muscardinus,* specimens from different localities were grouped together according to geographic region because of the

small sample size. In the present analysis four new populations of *Eliomys quercinus* are studied and compared with previously analysed populations (for details on localities and sample size see Filippucci et al., 1988 c and Vujošević et al., 1993). The sample size of Israeli populations of *Eliomys melanurus* was increased. Values of genetic variation and differentiation in *Eliomys* are here summarized for each chromosomal form.

Tab. 1 – Collecting site, number of specimens examined, and sample designation of each dormouse population analysed.

SPECIES	COLLECTING SITE	N. SPEC.	SAMPLE DESIGN
Myomimus rouchi	Sütlüce, Gelibolu, Thrace, Turkey	1	GEI
Myoxus glis	Tarvisio, Friuli, Italy	15	TAR
	Asiago, Venetia, Italy	100	ASI
	Viggianello, Mt. Pollino, Basilicata, Italy	2	POL
	Cerenzia, Sila Mts., Calabria, Italy	11	SIL
	Gambarie, Aspromonte Mts., Calabria, Italy	1	ASP
	Yenicckoy, Istranca Mts., Thrace, Turkey	2	IST
Muscardinus avellanarius	Asiago, Venctia, Italy	3	VEN
	Bocca di Serra, Venetia, Italy	1	VEN
	Fiera di Primiero, Trentino, Italy	1	TRE
	Hain, Hessen, Germany	1	HES
	Godovic. Slovenia	1	SLO
	Secoveljske Soline, Pirano, Slovenia	1	SLO
	Petkovica, Mt. Cer, Serbia	1	SER
	Mt. Scalambra. Latium, Italy	1	LAT
	Bellegra, Latium, Italy	1	LAT
	Avellino, Campania, Italy	3	CAM
	Muro Lucano, Basilicata, Italy	1	BAS
	Lago Ampollino, Calabria, Italy	1	CAL
Dryomys nitedula	Tarvisio, Friuli, Italy	9	TAR
	Asiago, Venetia, Italy	4	ASI
	Piani di Ruggio, Mt. Pollino, Basilicata, Italy	4	POL
	Idrija, Slovenia	1	IDR
	Boracko Jczero, Herzegovina	1	BOR
	Mt. Pelister, Macedonia	1	PEL
	Edirne, Thrace, Turkey	8	EDI
	Hurfesh, Upper Galilee, Israel	1	ISR
Eliomys quercinus (*)	Figueiras, Catalogna, Spain (2n=48)	15	FIG
	Affile and Cervara, Latiurn, Italy(2n=48)	4	LAT
	Pag Island, Dalmatia (2n=48)	2	PAG
	Koblenz, Germany (2n=50)	4	KOB
Eliomys melanurus (*)	Nahal Zin, Negev Desert, Israel (2n=48)	7	NAH
	Mizpe Ramon, Negev Desert, Israel (2n=48)	4	MIZ

(*) Other populations of this genus previously analysed electrophoretically (Filippucci et al., 1988 c and Vujoševićet al.. 1993) will be included in the present study and their results will be summarized according to geographic region and diploid chromosomal number: Iberian Peninsula: 2n=48 (QQ48); Italian Peninsula: 2n=48 (QP48); Balkan Peninsula: 2n=48 (QD48); Central Europe: 2n=50 (QQ50); Central and Eastern Alps: 2n=52 (QQ52); Western Alps: 2n=54 (QQ54); Israel: 2n=48 (MM48); Morocco: 2n=46 (MM46).

Genic variation of structural genes encoding for enzymatic and non-enzymatic protein was assessed using standard horizontal starch-gel electrophoresis. Tissues of each specimen were preserved in the laboratory at -80°C until required for processing. Homogenates for electroplioresis were obtained from portions of muscle or kidney tissue crushed in distilled water. All gels were prepared using an 11% suspension of Connaught hydrolyzed starch.

40-42 loci were analyzed, encoding for two non-enzymatic proteins and for 28 enzymes. The following loci were analyzcd: Alcohol dehydrogenase *(Adh;* E.C. 1.1.1.1: cathodal, kidney); α-Glycerophosphate dehydrogenase *(α-Gpdh*; E.C. 1.1.1.8; anodal, muscle), Sorbitol dehydrogenase *(Sdh;* E.C. 1.1.1.14; cathodal, kidney), Lactate dehydrogenases (E.C. 1.1.1.27; muscle; *Ldh-1*: anodal, and *Ldh-2:* cathodal), Malate dehydrogenascs (E.C. 1.1.1.37; muscle; *Mdh-I:* anodal, and *Mdh-2:* cathodal), Malic enzyme (E.C. 1.1.1.40; muscle; *Me-I* and *Me-2:* anodal), Isocitrate dehydrogenase (E.C. 1.1.1.42; muscle; *Idh-1* and *Idh-2*: anodal), 6-Phosphogluconate deliydrogenase (E.C. 1.1.1.44; muscle; *6-Pgdh*: anodal), Glucose-6-phosphate dehydrogcnase (E.C. 1.1.1.49; muscle; *G6pdh*, anodal), Glyceraldehyde-3-phosphate deliydrogenase (E.C. 1.2.1.12; muscle; *G3pdh*, anodal), Indophenol oxidase (1.15.1.1; muscle; *Ipo*, anodal), Nucleoside phosphorilase (E.C. 2.4.2.1; muscle: *Np,* anodal), Glutamate-oxaloacetate transaminase (E.C. 2.6.1.1 ; muscle; *Got-I:* anodal, and *Got-2:* cathodal), Hexokinase (E.C. 2.7.1.1; kidney; *Hk*, anodal), Creatine kinase (E.C. 2.7.3.2; muscle; *Ck.* anodal), Adenylate kinase (E.C. 2.7.4.3; muscle; *Adk,* anodal), Phosphoglucomutase (E.C. 2.5.7.1; muscle; *Pgm*, anodal), Esterases (E.C. 3.1.1.1; muscle: *Est-1, Est-2, Est-3,* anodal; in *Myoxus* an additional locus was also considered: *Est-4,* anodal), Peptidascs (E.C. 3.4.11; muscle; *Pep-1, Pep-2, Pep-3,* anodal), Aminopeptidases (E.C. 3.4.11; muscle; *Ap-1* and *Ap-2*, anodal), Leucyl aminopeptidase (E.C. 3.4.11; muscle; *Lap-1* and *Lup-2,* anodal), Alkaline phosphatase (E.C. 3.1.3.1; muscle; *Aph,* anodal), Acid phosphatase (E.C. 3.1.3.2; muscle; *Acph*, anodal), Adenosine deaminase (E.C. 3.5.4.4; muscle; *Ada,* anodal), Aldolase (E.C. 4.1.2.13; muscle, ***Aldo,*** anodal), Fumarase (E.C. 4.2.1.2; muscle; *Fum*, anodal), Mannose phosphate isomerase (E.C. 5.3.1.8; muscle; *Mpi*, anodal), Glucose phosphate isomerase (E.C. 5.3.1.9; muscle; *Gpi*, anodal in *Myoxus* and *Myomimus* and cathodal in *Dryomys*, *Muscardinus* and *Eliomys*), General Protein (muscle; *Pt-3* and *Pt-4*, anodal).

The electroplioretic techniques used were those described for *Eliomys* by Filippucci et al. (1988 c).

Isozymes were numbered in order of decreasing mobility from the most anodal. Allozymes were numbered according to their mobility relative to the commonest allele (=*100*) in the reference population of *Myoxus* from Asiago (in the comparisons among the dormouse populations and among the five genera), and of *Muscardinus* from Venetia (North Italy). In *Eliomys* and *Dryomys* the reference populations were those from Giazza (2n=52; sec Filippucci et al., 1988 c) and Tarvisio respectively. Allozymic data were analysed as genotype frequencies with the BIOSYS-1 program of Swofford & Selander (1981). Intrapopulational genetic variation was estimated using the mean heterozygosity per locus (expected: *He,* and observed: *Ho*), the proportion of polymorphic loci in the population (*P1%* and

P5%), and the average number of alleles per locus ***(A).***

The amount of genetic divergence between populations was estimated from the indices of standard genetic identity (*I*) and distance (*D*) proposed by Nei (1978). The high number of loci analysed compensates for the small sample size of some populations. Values of heterozygosity and genetic distances are therefore reliable with a reasonable margin of precision (Sarich, 1977; Nei, 1978; Gorman & Renzi, 1979; Sage et al., 1986). Dendrograms of the genetic relationships among populations and species were obtained using the unweighted pair group cluster analysis UPGMA (Sokal & Sneath, 1963).

RESULTS

GENETIC PATTERN

42 loci were analysed in ***Myoxus;*** 41 loci were analysed in *Myomimus*, *Muscardinus* and *Eliomys* and only 40 loci in *Dryomys*. Comparison among the five genera was carried out on the basis of 38 gene loci, Esterases being excluded from the analysis.

Myoxus - Twenty-five out of the 42 loci analysed (59%) were monomorphic and fixed for the same allele. The allelic frequencies at the polymorphic loci are given in Table 2. The highest number of polymorphic loci was observed in the population from North Italy ASI (14 loci), the lowest was observed in the Turkish population IST (3 loci).

Myomimus - The Turkish specimen displayed polymorphism only at the locus *Est-3* and 98% of the loci were monomorphic.

Muscardinus - 22 out of the 41 loci analysed (54%) were monomorphic and fixed for the same allele in the populations. The allelic frequencies at the polymorphic loci are given in Table 3. The highest number of polymorphic loci was observed in the populations from Venetia and Serbia (7 loci), the lowest was observed in the specimen from Calabria (1 locus).

Dryomys - Twenty out of the 40 loci analysed (50%) were monomorphic and fixed for the same allele in the populations considered. For allelic frequencies see Table 2 in Filippucci et al. (1995).

Eliomys - **A** total of 120 specimens of the garden dormouse have been analysed, representing 15 populations of *E. quercinus* and 3 populations of *E. melanurus*. Eighteen out of the 41 loci analysed (44%) were found to be monomorphic: *Ldh-2, Mdh-1, Mdh-2, Me-1, Idh-1, Idh-2, G3pdh, Ipo, Got-2, Ck,* ***Adk,*** *Est-3, Aph, Lap-1, Fum, Pgi, Pt-3, Pt-4*. Twenty-three loci were polymorphic and differentiated between the populations. In Table 4 allelic frequencies are given only for the new populations analysed (FIG, KOB, LAT, PAG, NAH, MIZ). For allelic frequencies in other populations of *E. quercinus* see Filippucci et al. (1988 c) and Vujošević et al. (1993).

GENETIC DIFFERENTIATION

Myoxus - The locus *Ap-2* was discriminant and the locus *Est-3* partially discriminant between the subspecies *M. g. italicus* and the populations from North Italy and Turkish Thrace. In addition, the loci *Adh* and *G6pdh* partially

discriminated the Turkish specimens from Italian populations. Differences in allelic frequencies at the loci *αGpdh* and *Pep-2* were observed between the North Italian populations from Tarvisio and Asiago.

Tab. 2 – Allelic frequencies observed at 17 of the 42 loci analysed in populations of *Myoxus glis*. For abbreviations of localities see Table 1.

LOCUS		TAR	ASI	POL	SIL	ASP	IST
ddh	*100*	1 00	1 00	1.00	1.00	1.00	0.25
	105	--	--	--	--	--	0.75
αGpdh	*96*	0 87	0 39	--	--	--	--
	100	0 13	0 61	1.00	1.00	1.00	1.00
Me-2	*97*	--	0 04	025	0.36	--	--
	100	0 83	0 86	0.75	0.64	1.00	1.00
	103	0 17	0 10	--	--	--	--
G6pdh	*96*	021	0 13	--	0.18	0.50	1.00
	100	0 79	0 87	1.00	0.82	0.50	--
Hk	*100*	0 88	0 80	1.00	1.00	1.00	1.00
	103	0 12	0 20	--	--	--	--
Pgm	*100*	100	0 99	1.00	1.00	1.00	1.00
	105	--	0 01	--	--	--	--
Ap-1	*100*	1 00	0 95	1.00	1.00	1 00	1.00
	105	--	0 05	--	--	--	--
Ap-2	*85*	1.00	1 00	--	--	--	1.00
	100	--	--	1.00	1.00	1.00	--
Pep-1	*100*	0 96	0 94	1.00	1.00	1.00	1.00
	110	0 04	0 06	--	--	--	--
Pep-2	*97*	0 43	0 17	--	--	--	--
	100	0 47	0 83	1.00	1.00	1.00	1.00
Pep-3	*96*	0 03	0 02	--	0.06	0.50	--
	100	0 97	0 98	1.00	0.94	0.50	1.00
Aph	*96*	0 23	0 06	--	0.25	0.50	--
	100	0 77	0 94	1.00	0.75	0.50	1.00
Ada	*100*	1 00	0 94	0.75	0.94	1.00	1.00
	104	--	0 06	0.25	0.06	--	--
Mpi	*100*	1.00	1 00	1.00	0.96	1.00	1.00
	105	--	--	0.04	--	--	--
Est.1	*100*	0 93	0 93	1.00	1.00	1.00	1.00
	108	0 07	0 07	--	--	--	--
Est-3	*100*	1.00	0 89	--	--	--	0.75
	104	--	0 11	1.00	1.00	1.00	0.25
Est-4	*96*	0 21	0 45	--	--	--	0.25
	100	0 79	0 55	1.00	1.00	1.00	0.75
	N	15	100	2	11	1	2
	He	0 062	0 070	0.024	0.036	0.071	0.036
	Ho	0 039	0 045	0.024	0.028	0.071	0.036
	PI%	0 238	0 333	0.048	0.143	0.071	0.071
	P5%	0 190	0 286	0.048	0.119	0.071	0.071
	A	1238	1357	1.047	1.143	1.071	1.071

The following loci were monomorphic and fixed for the same allele in all the populations: *Sdh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Me-1, Idh-1, Idif-2, 6Pgdh, G3pdh, Ipo, Np, Got-1, Got-2, Ck, Adk, Est-2, Acph, Aup-1, Lap-2, Aldo, Fum, Pgi, Pt-3,* and *Pl-4.*

I'ab. 3 – Allelic frequencies at 19 of the 41 loci analysed in *Muscardinus avellanarius*. For abbreviations of localities see Table 1.

LOCUS		VEN	TRE	LAT	CAM	BAS	CAL	SLO	SER	HES
αGpdh	*98*	--	--	--	--	0.50	--	--	--	--
	100	1.00	1.00	1.00	1.00	0.50	1.00	1.00	1.00	1.00
Sdh	*94*	--	--	1.00	1.00	1.00	1.00	--	--	--
	100	1.00	1.00	--	--	--	--	1.00	1.00	1.00
Me-1	*100*	0.63	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	102	0.37	--	--	--	--	--	--	--	--
Me-2	*97*	0.12	--	--	--	--	--	--	--	--
	100	0.76	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	102	0.12	0.50	--	--	--	--	--	--	--
Idh-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00	--	1.00	1.00
	105	1.00	--	--	--	--	--	1.00	--	--
Idh-2	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50
	105	--	--	--	--	--	--	--	--	0.50
6Pgdh	*95*	--	--	--	--	0.50	--	0.25	--	--
	100	1.00	1.00	1.00	1.00	0.50	1.00	0.75	1.00	1.00
G6pdh	*95*	0.50	--	--	--	--	--	--	--	--
	100	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hk	*90*	--	--	1.00	1.00	1.00	1.00	--	--	--
	100	1.00	1.00	--	--	--	--	1.00	1.00	1.00
Ap-1	*100*	0.88	1.00	1.00	1.00	1.00	0.50	0.25	1.00	1.00
	103	0.12	--	--	--	--	0.50	0.75	--	--
Pep-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00
	105	--	--	--	--	--	--	--	0.50	--
Pep-2	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00
	105	--	--	--	--	--	--	--	0.50	--
Lap-2	*96*	0.25	--	0.25	0.08	0.50	--	--	--	--
	100	0.75	1.00	0.75	0.92	0.50	1.00	1.00	1.00	1.00
Aph	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00
	105	--	--	--	--	--	--	--	0.50	--
Ada	*90*	0.12	0.50	--	--	0.50	--	--	0.50	--
	100	0.88	0.50	1.00	1.00	0.50	1.00	1.00	0.50	1.00
Mpi	*90*	--	--	--	0.08	--	--	--	--	--
	100	1.00	1.00	1.00	0.92	1.00	1.00	1.00	0.50	1.00
	105	--	--	--	--	--	--	--	0.50	--
Est-1	*100*	0.63	1.00	0.25	--	--	--	0.50	0.50	0.50
	104	0.37	--	0.75	1.00	1.00	1.00	0.50	0.50	0.50
Est-2	*100*	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	1.00
	103	--	--	--	--	--	--	0.50	--	--
Dt-3	*96*	--	--	--	--	--	--	--	0.50	--
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*0.50*	1.00
	N	4	1	2	6	1	1	2	1	1
	He	0.078	0.049	0.024	0.008	0.098	0.024	0.057	0.171	0.049
	Ho	0043	0.040	0.024	0.008	0.098	0.024	0.049	0.171	0.049
	P1%	0.171	0.049	0.049	0.049	0.098	0.024	0.098	0.171	0.049
	P5%	0.171	0.049	0.049	0.049	0.098	0.024	0.098	0.171	0.049
	A	1.200	1.049	1.049	1.049	1098	1.024	1098	1.171	1049

The following loci were monomorphic and fixed for the same allele in all the populations: *Adh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, G3pdh, Ipo, Np, Got-1, Got-2, Ck, Adk, Pgm, Acph, Ap-2, Pep-3, Lap-1, Aldo, Fum, Pgi, Pt-3,* and *Pt-4.*

Tab. 4 – Allelic frequencies observed at 17 of the 41 loci analysed in populations of *Eliomys quercinus* and *E. melanurus*. For abbreviations of localities see Table 1.

LOCUS		*E quercinus*				*E melanurus*	
		FIG 2n=48	KOB 2n=50	PAG 2n=48	LAT 2n=48	NAH 2n=48	MIZ 2n=48
Adh	*90*	1 00	--	--	0.63	--	--
	95	--	--	--	--	0.14	--
	100	--	1.00	1.00	0.37	--	--
	105	--	--	--	--	0.86	1.00
αGpdh	*100*	0 93	0 90	1.00	1.00	0.79	1.00
	103	0 07	0 10	--	--	--	--
	105	--	--	--	--	0.21	--
Sdh	*100*	1.00	1 00	1.00	1.00	--	--
	106	--	--	--	--	1.00	1.00
1.dh-1	*94*	--	--	--	--	1.00	1.00
	100	1 00	1.00	1.00	1.00	--	--
Me-2	*100*	0 82	0 80	1.00	0.50	1.00	0.87
	102	0 18	0 20	--	0.50	--	0.13
6Pgdh	*100*	0 57	1.00	1.00	1.00	--	--
	108	0 43	--	--	--	1.00	1.00
G6pdh	*95*	--	--	--	--	0 07	--
	100	1 00	1.00	1.00	1.00	0.93	1.00
Np	*95*	0 38	--	0.25	--	--	--
	100	0 50	1.00	--	1.00	1.00	1.00
	106	0 12	--	--	--	--	--
	110	--	--	0.25	--	--	--
	120	--	--	0.50	--	--	--
Pgm	*100*	0 91	1 00	1.00	1.00	0 93	0.75
	104	0 03	--	--	--	0.07	0.25
Pep-2	*100*	--	--	1.00	1.00	--	--
	105	1 0 0	1.00	--	--	1.00	1.00
Pep-3	*100*	0 65	0 80	1.00	1.00	0.93	0.88
	106	0 35	020	--	--	0.07	0.12
Ap-1	*100*	--	--	1.00	--	0.93	0.88
	104	1.00	1.00	--	1.00	0.07	0.12
Ap-2	*95*	--	--	--	--	0.07	0.12
	100	0 43	0 60	1.00	1.00	0.93	0.88
	105	0 57	0 40	--	--	--	--
Ada	*90*	--	0 20	--	--	--	--
	100	0 90	0 60	0.50	0.50	--	--
	105	0 10	0 20	0.50	0.50	--	--
	110	--	--	--	--	1.00	1.00
Mpi	*90*	--	--	--	--	1.00	1.00
	100	1.00	1.00	1.00	1.00	--	--
Est-1	*100*	0 73	020	--	0.88	--	--
	105	0 27	080	1.00	0.12	0.93	0.88
	108	--	--	--	--	0.07	0.12
Est-2	*100*	--	0 40	--	0.75	1.00	0.88
	105	100	0 60	1.00	0.25	--	0.12
	N	15	4	2	4	7	4
	He	0 078	0 072	0.037	0.068	0.036	0.047
	Ho	0 063	0 059	0.037	0.049	0.038	0.049
	PI%	0 219	0 171	0.049	0.122	0.195	0.171
	P5%	0 195	0 171	0.049	0 122	0.195	0.171
	A	1.244	1 195	1.073	1.122	1.195	1.171

Tab. 5 – Electromorphs observed in the five genera of Myoxidae.

LOCUS	*Myoxus*	*Myomimus*	*Dryomys*	*nitedula*	*Muscardinus*	*Eliomys quercinus*						*E. melanurus*	
	glis	*roachi*	**Europe**	Israel	*avellanarius*	QQ48	QP48	QD48	QQ50	QQ52	QQ54	MM48	MM46
Adh	100, 105	96	106.103	106.103	90	102	92.102	102	102	102.108	102. 108, 110	97. 108	108
a-Gpdh	96, 100	92	90, 95	90	102, 104	110, 113	110	110	110. 113	94, 110, 113	94, 110, 113	110, 115	110
Sdh	100	92	106	106	104, 110	92	92	92	92	92	92	98	98
Ldh-1	100	100	85, 102	104	102	96	96	96	96	96.98	96	90	80. 90. 93. 96
Ldh-2	100	100	100	100	100	100	100	100	100	100	100	100	100
Mdh-1	100	90	95,100	100	100	100	100	100	100	100	100	100	100
Mdh-2	100	100	103	103	100	100	100	100	100	100	100	100	100
Me-1	100	80	94	94	88, 90	96	96	96	96	96	96	96	96
Me-2	97, 100, 103	90	90, 92	87, 90	83, 86, 88	96, 98	96	96, 98	96, 98	96.98	96, 98	96, 98	96, 98
Idh-1	100	104	106	106	77, 82	80	80	80	80	80	80	80	80
Idh-2	100	90	97. 105	97, 105	88, 93	85	85	85	85	85	85	85	85
6Pgdh	100	95	104	104, 108	100, 95	90,98	90, 98	90	98	98	98	98	102
G6pdh	96,100	112	104,108	98	97,102	94	94	94	94	94	94	89, 94	94
G3pdh	100	90	90	90	104	94	94	94	94	94	94	94	94
Ipo	100	110	105	105	94	100	100	100	100	100	100	100	100
Np	100	70	88, 93	93	96	50, 55, 62	50, 55, 75	50, 65, 75	50, 55	50, 55	50, 55	55	50, 55
Got-1	100	94	94, 104	104	106	97	97	97, 102	97	91	97	97	91
Got-2	100	100	100	100	100	100	100	100	100	100	100	100	100
Hk	100,103	86	90	90, 93	92, 102	97	94, 97	97	94, 97	94, 97	94, 97	97	97
Ck	100	92	92	92	100	100	100	100	100	100	100	100	100
Adk	100	100	104.106	104	98	100	100	100	100	100	100	100	100
Pgm	100, 105	100	106	106	104	104, 108	104, 108	104	104, 108	97, 104, 108	97, 104, 108	104, 108	104
Aph	96, 100	98	93, 95	93	92, 97	82	82	82	82	82	82	82	82
Acph	100	95	95	95	92	100	100	100	100	100, 105	100	100	100
Pep-1	100. 110	118	118	116	120, 125	115	113, 115	113, 115	115	113, 115	113, 115	115	113, 115
Pep-2	97, 100	95	85	85	104	95	90	90	90, 95	90	90	95	95
Pep-3	96, 100	93	90	90	86	93, 98	93, 98	93	93, 98	93, 98	93, 98	95	95
Ap-1	100, 105	125	116, 120, 123	120	115, 118	110	110	106	110	106, 110	106, 110	106, 110	106, 110
Ap-2	85, 100	135	115	115	125	90, 95	90	90	90, 95	90	90	83, 90	90, 95
Lap-1	100	98	96	96	90	94	94	94	94	94	94	94	94
Lap-2	100	73	80, 87	83	98, 102	86	86	86	86	82,86	86	86	86
Ada	100, 104	106	63, 68	68	75, 85	60, 65	45, 60, 65, 73	60, 65	50, 60, 65	50, 60, 65,73	60, 65	70	55, 70
Aldo	100	100	98, 103	103	90	92	92	92, 96	92, 96	92	92, 96	92	92, 96
Fum	100	104	96	96	98	94	94	94	94	94	94	94	94
Mpi	100, 105	83	95	95	66, 76, 81	90	90	90	90	90	90	80	80, 90
Pgi	100	95	-85	-85	-85	-80	-80	-80	-80	-80	-80	-80	-80
Pt-3	100	104	96	96	106	98	98	98	98	98	98	98	98
P1-4	100	94	94	94	100	100	100	100	100	100	100	100	100

Muscardinus - The loci *Sdh* and ***Hk*** were found to be discriminant and the locus *Est-1* was partially discriminant between populations from Central-Southern Italy and the other European populations. **A** new allele for *Idh-1* was found fixed in specimens from Slovenia.

Dryomys - **A** high differentiation was observed between European and Israeli populations, with four loci (*Ldh-1*, *G6pdh*, *Pep-1*, *Lap-2)* being discriminant between the two groups.

Eliomys - Five loci *(Adh, Sdh, Ldh-1, Ada,* and *Mpi)* displayed fixation or predominance of alternative alleles, allowing European populations of *E. quercinus* to be distinguished from North African and Israeli populations of *E. melanurus.* The Moroccan population of *E. melanurus* can be distinguished from Israeli and European populations by having fixed alternative alleles for *6Pgdh, Est-2,* and *Pep-3* (see Filippucci et al., **1988** c). Among European populations of *E. quercinus*, characterized by four different karyotypes, there is no locus fixed for alternative alleles. Nevertheless, few loci are partially discriminant.

Intergeneric differentiation - **A** comparison among the five genera was conducted on **38** gene loci: *Adh, a-Gpdh, Sdh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Me-1, Me-2, Idh-1, Idh-2, 6Pgdh, G6pdh, G3pdh, Ipo-2, Np, Gut-I, Got-2,* ***Hk,*** *Ck, Adk, Pgm, Aph, Acph, Pep-1, Pep-2, Pep-3, Ap-I, Ap-2, Lap-1, Lap-2, Ada, Aldo, Fum, Mpi, Pgi, Pt-3,* and *Pt-4.* Designation of electromorph mobilities for **38** loci is given in Table 5. **In** this analysis each allozyme was numbered according to its mobility relative to the commonest allele (=100) in the reference population of *Myoxus* from Asiago.

Only two loci *(Ldh-2* and *Got-2)* were found monomorphic and fixed for the same allele **in** the five genera. Therefore the five genera are highly differentiated.

A total of 252 alleles was observed at **38** loci in the five genera (Esterases were excluded from this analysis). The highest number of alleles (14) was observed at the locus *Adu.* The number of alleles observed in each genus is given in Table 6.

Tab. 6 – Number of coinmon and exclusive alleles observed in each genus of Myoxidae. The total number of alleles observed at **38** common loci was 252.

NLIMBER OF ALLELES IN COMMON BETWEEN GENERA					TOTAL N ALLELES (*)	N EXCLUSIVE ALLELE (*)	N. ALLELES ESTERASES
	Myoxus	*Myomimus*	*Muscardinus*	*Dryomys*			
Myoxus	--				51	37	7
Myomimus	7	--			38	23	**4**
Muscardinus	7	4	--		53	41	**6**
Dryomys	3	8	5	--	61	*50*	**6**
Eliomys	11	7	7	3	75	60	**8**

(*) calculated on 38 loci: being Esterases excluded from intergeneric comparison

The highest percentage of exclusive alleles was observed in *Dryomys* (84%; 50 alleles) and in *Eliomys* (80%; 60 alleles), whereas the lowest was observed in *Myomimus* **(61%;** 23 alleles). In *Myoxus* 72% (37 alleles) and in *Muscardinus* 77% (41 alleles) of the alleles were exclusive. The maximal affinity was found between *Myoxus* and *Eliomys,* having the highest number (11) of common alleles.

GENETIC **VARIATION**

Levels of genetic variation within populations of *Myoxus glis, Muscardinus avellanarius*, and new populations of the genus *Eliomys* are given in Tables 2, **3,** and 4 respectively. In Table 7 the mean values of genetic variation are given for each species. The observed values of genetic variation are within the range generally reported for other rodents (Selander, 1976; Nevo et al., 1984, 1990). The mean values of observed heterozygosity ranged from 0.024 in *Myomimus* to 0.062 **in** *Dryomys.*

Tab. 7 – Mean values of genetic variation observed in six species of Myoxidae.

SPECIES	N. POP.	N. SPEC.	N. LOCI	*HE*	*HO*	*A*	*P1%*	*P5%*
M. glis	6	131	42	0.050	0.040	1.154	0.151	0.131
M. avellanarius	9	19	41	0.061	0.057	1.080	0.056	0.056
M. roachi	I	I	41	0.024	0.024	1.024	0.024	0.024
D. nitedula	8	29	40	0.063	0.062	1.112	0.109	0.109
E. quercinus								
QQ54 (*)	2	24	41	0.066	0.064	1.357	0.317	0.292
QQ52 (*)	4	27	41	0.050	0.045	1.439	0.341	0.292
QQ50	2	12	41	0.069	0.067	1.195	0.183	0.183
QP48	3	21	41	0.068	0.054	1.341	0.268	0.268
QQ48	2	16	41	0.051	0.056	1.146	0.134	0.122
QD48	2	5	41	0.053	0.047	1.195	0.097	0.097
	Average			0.059	0.055	1.279	0.223	0.209
Eliomys melanurus								
MM48	2	11	41	0.041	0.043	1.244	0.183	0.183
MM46 (*)	1	4	41	0.094	0.061	1.293	0.244	0.244
	Average			0.067	0.052	1.268	0.213	0.213

(*) Data from **Filippucci** et al. (1988 c).

GENETIC DISTANCE

Nei's (1978) values of genetic identity *(I)* and distance (*D*) were calculated among populations, species, and genera for all pairwise comparisons from the allelic frequencies at 38-42 loci tested (Table **8).**

Tab. **8** – Values of Nei's (1978) unbiased genetic distance (below diagonal) and identity (above diagonal) observed among: 1) populations of *Myoxus* (42 loci); *2)* populations of *Muscardinus* (41 loci); 3) populations of *Dryomys* (40 loci); 4) chromosomal forms of *Eliomys* (41 loci); 5) gcncra **(38** loci).

1) *Myoxus*	TAR	ASI	POL	SIL	ASP	IST
TAR	--	0.989	0.922	0.922	0.914	0.946
ASI	0.011	--	0.946	0.943	0.930	0.964
POL	0.081	0 055	--	1.000	0.982	0.926
SIL	0.081	0.059	0.000	--	0.989	0.930
ASP	0.090	0.072	0.019	0.011	--	0.932
IST	0.055	0.036	0.076	0.073	0.070	--

2) *Muscardinus*	ASI	TRE	LAT	CAM	RAS	CAL	SLO	SER	HES
ASI	--	0.983	0.940	0.931	0.911	0.926	0.953	0.953	0.983
TRE	0.017	--	0.925	0.913	0.899	0.907	0.941	0.955	0.975
LAT	0.062	0.078	--	1.000	0.981	0.994	0.910	0.910	0.944
CAM	0.072	0.091	0.000	--	0.977	0.994	0.904	0.906	0.938
BAS	0.093	0.107	0.019	0.023	--	0.969	0.876	0.889	0.911
CAL	0.077	0.098	0.006	0.006	0.032	--	0.916	0.898	0 932
SLO	0.048	0.061	0.095	0.101	0.132	0.087	--	0.920	0 954
SER	0.049	0.046	0.094	0.099	0.118	0.107	0 084	--	0 955
HES	0.017	0.025	0.058	0.064	0.093	0.071	0 047	0.046	--

3) *Dryomys*	POL	ASI	TAR	IDR	BOR	PEL	EDI	ISR
POL	--	0.967	0.973	0.944	0.967	0.955	0.954	0.847
ASI	0 034	--	0.979	0.995	0.944	0.920	0.946	0.851
TAR	0 027	0.021	--	0.970	0.968	0.948	0.964	0.858
IDR	0 057	0.005	0.030	--	0.936	0.909	0.947	0.830
BOR	0 034	0.058	0.033	0.066	--	0.961	0.973	0.824
PEI	0 046	0.083	0.053	0.095	0.040	--	0.950	0.808
EDI	0 047	0.056	0.037	0.054	0.027	0.052	--	0.797
ISR	0 166	0.161	0.153	0.186	0.194	0.213	0.227	--

4) *Eliomys*	QQ54	QQ52	QQ50	QQ48	QP48	QD48	MM48	MM46
Q54	(0.013)	0.980	0.917	0.894	0.956	0.920	0.788	0.779
QQ52	0 021	(0009)	0910	0892	0951	0.939	0.791	0.779
QQ50	0 086	0095	(0015)	0.961	0934	0.931	0.807	0.815
QQ48	0 112	0 115	0040	(0061)	0924	0.911	0.802	0.819
QP48	0 045	0051	0069	0079	(0019)	0.917	0.794	0.795
QD48	0 084	0063	0072	0094	0087	(0.002)	0.799	0.765
MM48	0 238	0235	0215	0220	0230	0.224	(0.000)	0.885
MM46	0 250	0250	0205	0200	0230	0.267	0.122	--

5)	*Myoxus*	*Myomimus*	*Muscardinus*	*Dryomys*	*Eliomys*
Myoxus	--	0 190	0 195	0.083	0.277
Myomimus	1660	--	0 084	0.166	0.162
Muscardinus	1633	2 475	--	0.124	0.196
Dryomys	2 479	1.811	2 086	--	0.112
Eliomys	1283	1 825	1628	2.478	--

Myoxus - The values of *I* and *D* were calculated from the allelic frequencies at 42 loci. The highest values of genetic distance were observed comparing populations of M. *glis italicus* from Southern Italy with other populations: *D* = 0.073, ranging from 0.055 to 0.090. **An** UPGMA dendrogram summarizing the genetic relationships found between the populations studied is given in Fig. 1.

Muscardinus - The values of genetic distance and identity were obtained from

the allelic frequencies at 41 loci. Populations from Central and Southern Italy, attributed to the subspecies *M. a. speciosus*, displayed a mean value of genetic distance $D = 0.090$ in comparison with other European populations. An UPGMA dendrogram summarizing the genetic relationships found between the populations studied is given in Fig. 2.

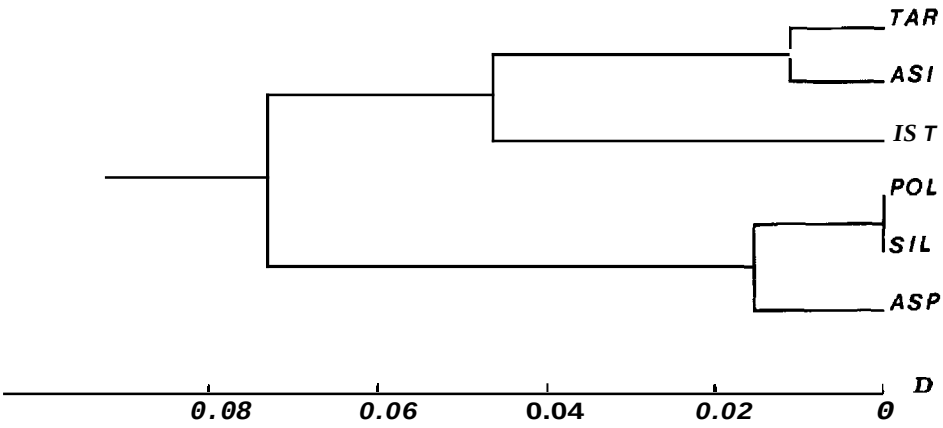

Fig. 1 – UPGMA dendrogram summarizing the genetic relationships among the studied populations of *Myoxus glis*. D: Nei's (1978) unbiased genetic distance, based on 42 loci. The cophenetic correlation coefficient is 0.952.

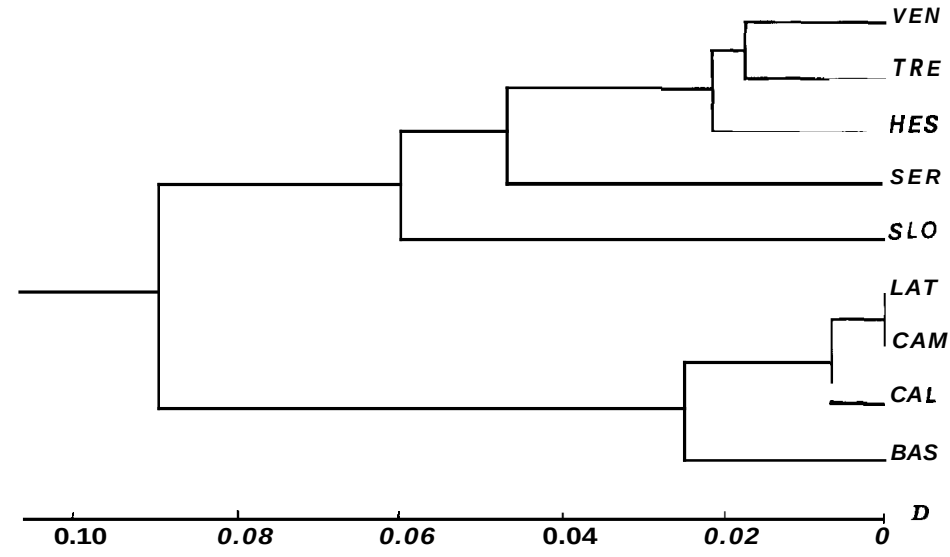

Fig. 2 – UPGMA dendrogram summarizing the genetic relationships among the studied populations of *Muscardinus avellanarius*. D: Nei's (1978) unbiased genetic distance, based on 41 loci. The cophenetic correlation coefficient is 0.904.

Dryomys - The values of D and I were calculated from the allelic frequencies at 40 loci. The two populations of *D. n. intermedius* from Asiago and Tarvisio showed a low value for genetic distance: $D = 0.021$. This value is higher than that found in the populations of *Myoxus* from the same localities. This fact can be related to the stenoecious behaviour of *Dryomys*, strictly linked with conifer and beech woods (Paolucci et al., 1987). The value of genetic distance between *D. n. intermedius* and *D. n. aspromontis* was also low, $D = 0.030$. The value for genetic distance between the Israeli population and the European populations of *D. nitedula* was comparatively high: $D = 0.186$. The UPGMA dendrogram summarizing the genetic relationships found between the populations studied is given in Fig. 5 of Filippucci et al. (1995).

Eliomys - The populations studied were assembled into two distinct groups: *E. quercinus,* including all the European populations, and *E. melanurus,* including North African and Israeli populations. The mean value of genetic distance between the two groups is $D = 0.231$. The genetic distance between Israeli (MM48) and Moroccan (MM46) populations of *E. melanurus* is $D = 0.122$.

Values for genetic distance among European groups are lower. Populations from the western Alps (2n = 54) displayed low values of genetic distance in comparisons with populations from the Central-eastern Alps (2n = 52): $D = 0.021$. The peninsular populations of *E. q. pallidus* (2n = 48) displayed higher values for genetic distance in comparison with Alpine populations: $D = 0.048$. Central European populations (2n = 50) are closer to Spanish populations with 2n = 48 ($D = 0.040$) than to Italian populations from the Alps ($D = 0.090$) or from the peninsula ($D = 0.069$) and to Dalmatic populations ($D = 0.072$). The lowest value of genetic distance for the Yugoslavian populations of *E. q. dalmaticus* (2n=48) was found in comparison with Alpine populations with 2n = 52 ($D = 0.063$), whereas $D = 0.094$ in comparison with 2n = 48 from Spain and $D = 0.087$ in comparison with *E. q. pallidus*. The UPGMA dendrogram summarizing the genetic relationships among the chromosomal forms of *Elionzys* is given in Fig. 3.

Tntergeneric comparison - Values for genetic distance and identity were calculated from the allelic frequencies at 38 loci. The highest value for genetic distance was found comparing *Dryomys* and *Myomimus* with the other three genera ($D = 2.251$). The lowest value for genetic distance was found in the comparison between *Myoxus* and *Elionzys* ($D = 1.283$). The UPGMA dendrogram summarizing the genetic relationships among the five genera studied is given in Fig. 4.

DISCUSSION

The mean values for genetic distances observed among the Italian subspecies of *Myoxus, Muscardinus* and *Eliomys* correspond to those generally observed among other rodent subspecies (Zimmerman et al., 1978; Graf, 1982; Filippucci et al., 1991). In these three genera the mean values for genetic distances were similar in the comparison between southern and northern Italian subspecies ($0.05 < D < 0.08$), indicating that the separation of the peninsular populations occurred in approximately the same period, about 0.3 - 0.4 m.y.B.P., according to Nei's (1975)

index of time of evolutionary divergence. This evaluation based on electrophoretic data corresponds to the fossil records, excluding a single sample of *Muscardinus avellanarius*, determined on the basis of a single tooth and found in a fissure filling of Gargano in Apulia of latest Villafranchian age (about 1.0 m.y.B.P.). *Myoxus glis* is known for the peninsula in late Galerian faunas (about 0.5 m.y.B.P.), whereas *Muscardinus avellanarius* and ***Eliomys*** *quercinus* are known in Rianian faunas (about 0.3 - 0.2 m.y.B.P.).

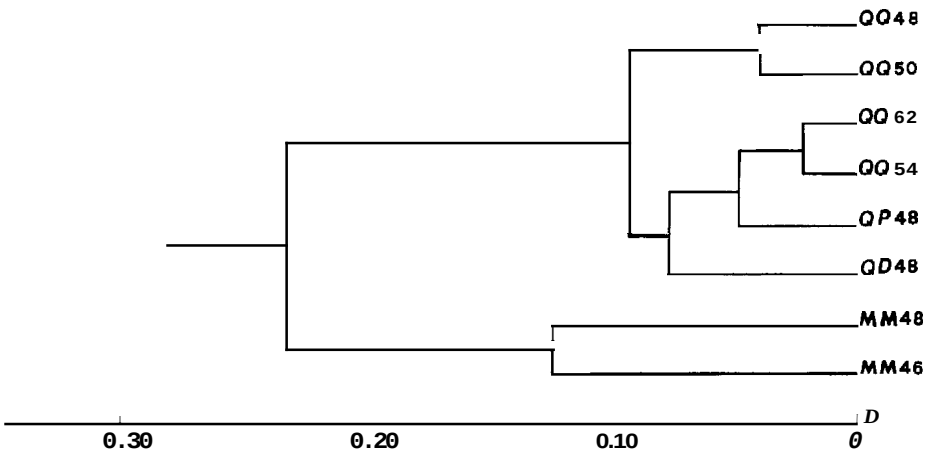

Fig. 3 – UPGMA dendrogram summarizing the genetic relationships among chromosomal forms of *Eliomys quercinus* and *E. melanurus*. D: Nei's (1978) unbiased genetic distance, based on **41** loci. The cophenetic correlation coefficient is 0.977.

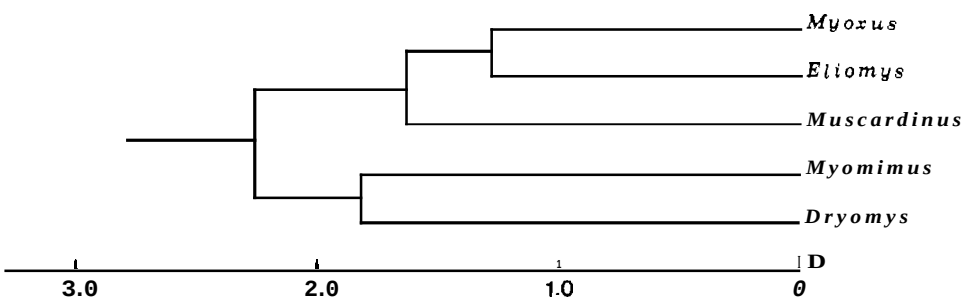

Fig. 4 – UPGMA dendrogram summarizing the genetic relationships among the five genera of Myoxidae. D: Nei's (1978) unbiased genetic distance, based on 38 loci. The cophenetic correlation coefficient is 0.979.

In *Dryomys nitedula*, the mean genetic distance observed between the two Italian subspecies was lower ($D = 0.03$), corresponding to values generally observed between local populations. This value confirms the recent origin of South Italian populations, attributed to the subspecies *aspromontis* von Lehmann, 1964.

This result supports the hypothesis of Roesler & Witte (1968) that *Dryomys* reached Southern Italy only recently, spreading from Alps to Calabria through the Appenines. **A** fossil record of *Dryomys nitedula* has been found in central Italy by Kotsakis (1991) in "Middle Würm" deposits, corresponding to the Isotopic Stage 3 (between 0.035 and 0.065 m.y.B.P.).

Fossil remains from Israel (Late Pleistocene) have been assigned to *D. nitedula* (Tchernov, 1988). The Israeli population of *Dryomys nitedula* can be differentiated from the Europeans having four discriminant loci and a high mean genetic distance: $D = 0.186$, based on 40 loci. This kind of value for genetic distance is generally observed at different stages of evolutionary divergence, usually associated with closely related, sibling species, especially in vertebrates (Avise & Aquadro, 1982). According to Thorpe (1982) and Nei (1987), if allopatric populations of dubious status have genetic distances (D) higher than 0.16, it is improbable that they are conspecific. The Israeli population could represent a separate species of *D. nitedula* and this hypothesis is supported by biometrical analysis (Filippucci et al., 1995), as well as by ecological and biological characteristics described by Nevo & Amir (1968). Such a conclusion should be confirmed by analysis of eastern populations of this species (Caucasian and Iranian).

In *Myoxus glis*, a low value for genetic distance ($D = 0.011$) was found between the North Italian populations of Tarvisio and Asiago. Nevertheless, differences in allelic frequencies were observed at two loci. The population from Asiago displayed a low frequency for the alleles 96 of *αGpdh* and 97 of ***Pep-2***, that are instead the most common in Tarvisio. These alleles are not present in populations of *M. g. italicus* nor in the sample from Turkish Thrace. This observation could indicate that mixed populations of *M. g. glis* and *M. g. postus* are present in North-eastern Italy. Mixed populations of these two taxa are already known for western Istria (Storch, 1978).

In the genus *Eliomys*, high values for genetic distance were observed between European and North African + Middle Eastern populations ($D = 0.231$, ranging froin 0.20 to 0.27), confirming the presence of two distinct lineages: *Eliomys quercinus* and *E. melanurus*. According to current estimates of evolutionary divergence time from genetic distance data (Nei, 1975), these two lines derived from a common ancestor about 1.2 m.y.B.P. Within each lineage, speciation events are still ongoing, as indicated by the presence of different karyotypes. Within *E. melanurus*, the genetic distance between the taxa *munbyanus* from Morocco and *melanurus* from Israel is relatively high ($D = 0.122$), suggesting a separation from a common ancestor about 0.6 m.y. B.P. This evaluation is in agreement with the fossil record. According to Kowalski & Rzebik-Kowalska (1991), the present-day species of the garden dormouse has been present in the Maghreb region only since the Middle Pleistocene and does not have its ancestor among the Miocene and Pliocene forms from Africa. Following Mein & Pickford (1992), the first occurrence of the genus *Eliomys* in Tunisia must be placed at about 0.5 m.y.B.P. Unfortunately fossil remains of *Eliomys* in the Middle East are known only from the Late Pleistocene of Israel (Tchernov, 1988). Other North African taxa (*cyrenaicus*, *denticulatus*, *tunetae*, and *occidentalis)* should be analysed to clarify

the phylogenetic relationships among subspecies of *Eliomys melanurus* and to assess their taxonomic status.

The mean values for genetic distance among European populations of *Eliomys quercinus,* characterized by 4 karyotypes, are lower, ranging from 0.021 (between alpine populations with 2n = 54 and 2n = 52) to 0.115 (between alpine populations with 2n = 52 and Spanish populations with 2n = 48), suggesting recent separation from a common ancestor in the last 500 000 years. Within *E. quercinus*, the most ancient populations are those from southern Europe with 2n = 48, as indicated by fossil records. This species disappeared from central Europe during the cold glacial periods of the Late Pleistocene. In France *E. quercinus* is present in many fossil assemblages of the Late Pleistocene (Chaline, 1974, 1977) and also in the late Pleistocene of Poland (Nadachowski, 1990). Central Europe was recolonized during the Holocene by Spanish, Italian and Dalmatic populations, characterized by chromosomal rearrangements, that spread northward from their refuge areas. Both karyologic and allozymic evidence suggest that the recolonization occurred with two flows of migrations. The western flow originated from the Iberian peninsula spreading eastwards and produced central European populations with 2n = 50. The eastern flow originated from Italy or the Balkan peninsula and generated the Alpine populations that increased westward their chromosome number to 2n = 54.

The genus is generally considered the upper taxonomic limit for application of electrophoretic analysis (Avise, 1974) and intergeneric comparisons are not very frequent. Mean values of D between genera of the same family were calculated for some groups of fishes, amphibians, and mammals (Avise & Smith, 1977; Hedgecock & Ayala, 1974; Graf, 1982; Honeycutt & Williams, 1982). These values were generally close to or higher than $D = 1$. In Arvicolidae, Graf (1982) found $D = 0.75$, but this low value is related to the recent origin of the Arvicolidae: 5 million years according to Chaline & Graf (1988). In Lagomorphs, Grillitsch et al. (1992) found D = 0.73 between *Lepus* and *Oryctolagus* and $D = 1.43$ between Leporidae and Ochotonidae.

The mean genetic distances observed in intergeneric comparisons among Myoxidae are high, corresponding to those observed in other vertebrates, and confirm the ancient origin of the five genera. The genera *Myoxus, Muscardinus, Myomimus*, and *Eliomys* were in fact already present in the Miocene (Storch, 1978; Chaline & Mein, 1979; Daams, 1981). *Myoxus* is considered the oldest genus and fossils belonging to this genus date from the Middle Oligocene and Early Miocene. The stratigraphically earliest named *Myoxus* species is *M. guerbezi* (Unay, 1990) from Kocayarma in Turkey (Middle Oligocene), followed by three other species of the Early Miocene: *M. apertus* (Mayr, 1979) from Weissenburg in southern Germany, *M. truyolsi* (Bruijn) from Spain and *M. major* (Bruijn) from Sardinia (Storch, 1978). The genus *Muscardinus* was present in the Middle Miocene with *M. thaleri* Bruijn in Spain (Daams, 1985). The genus *Myomimus* was also present in the Middle Miocene with the species *M. dehmi* (Bruijn) in Spain (Daams et al., 1988). The genus *Elionzys* is known from the late Middle Miocene with the species *E. truci* Mein & Michaux in Spain (Daams et al., 1988). *Dryomys* is considered the most recent genus. With the exception of a

Dryomys sp. from the Early Pliocene of Poland (Nadachowski, 1990), the oldest records are of Middle Pleistocene age (Janossy, 1962; Storch, 1975; Horaček, 1987) in central Europe and Chios island (Greece) and were assigned to *D. nitedula* or *D.* cfr. *nitedula*. According to Spitzenberger (1976), even though *D. nitedula* represents the rarest of the five European dormouse species in the fossil record, this genus cannot be considered so recent if we look at the sympatric occurrence in Taurus of *D. nitedula* and *D. laniger*, endemic to Turkey, and at its distribution range. Moreover, whereas *Eliomys*, ***Myoxus,*** and *Muscardinus* are typical of the western palaearctic region, *Dryomys* is the only genus with a wide but fragmentary distribution, ranging from eastern Switzerland and Italy to Tien Shan Mountains of Sinkiang (China). The electrophoretic data support Spitzenberger's hypothesis that the genus *Dryomys* is the most differentiated, having 84 % of exclusive alleles. Following Daams (1981) the fossil remains collected in the Middle and Late Miocene of Maghreb and ascribed to the genus *Afrodryomys* Jaeger must be ascribed to the genus *Dryomys*. In our opinion this position is for the moment only a working hypothesis.

Several attempts to elucidate phylogenetic relationships among dormouse genera were performed by several recent authors on morphological characters from paleontological as well as neontological evidences (de Bruijn, 1967; Kratochvil, 1973: Chaline & Mein, 1979; Daams, 1981; Rossolimo & Pavlinov, 1985; Wahlert et al., 1993; von Koenigswald, 1993). Nevertheless, the phylogeny of the Myoxidae is still controversial. The present study is the first attempt to investigate the phylogenetic relationships of Myoxidae by genetic data. According to the electrophoretic results, the highest affinity is found between *Myoxus* and *Eliomys*, sharing 11 alleles and displaying the lowest mean value of genetic distance ($U = 1.28$). *Dryomys* and *Myomimus* display instead the highest values of genetic distance when compared with the other genera.

The discrepancy observed between genetic and morphological data in reconstructing phylogenetic relationships among the Myoxidae is probably due to the ancient origin and separation of its genera. In taxa with a high level of differentiation, homoplasy can be extensive and divergence among these lineages can exceed the limits of resolution of isozyme electrophoresis (Murphy et al., 1990). According to Nei (1987), if genetic distance is greater than 1.0, the variance of D is large even if numerous loci are assayed. Hillis & Moritz (1990) instead consider electrophoresis an appropriate and effective method for reconstructing phylogenies of groups that, like the Myoxidae, evolved in the time frame of 5-50 million years before present.

CONCLUSIONS

In intra- and interspecific comparisons, a good correspondence is found between divergence time calculated from Nei's values of genetic distance and fossil evidence. Therefore, electrophoresis is considered a good and reliable tool in reconstructing phylogeny of the Myoxidae at subspecific and specific level. However, its use is questionable at generic level, as its results contradict evidence derived from different methods. A confirmation of phylogenetic relationships

among the Myoxidae should be provided by other molecular techniques that are more sensitive and powerful in reconstructing phylogenies at taxonomic levels higher than species.

ACKNOWLEDGEMENTS – The authors are grateful to Dr. G. Aloise, Dr. S. Baasner, Dr. M. Cagnin, Prof. M. Cristaldi, Dr. D. Franco, Dr. B. Kryštufek, Prof. G. Kurtonur, Dr. H. Meinig, Dr. B. Ozkan, Mr. P. Paolucci, and Dr. S. Simson for providing several dormice specimens. This research was supported by grants from C.N.R. ("Tassonomia ed evoluzione dei micromammiferi della penisola balcanica") and M.U.R.S.T. ("Ricerche faunistiche nel Mediterraneo occidentale"; "Genetica ecologica").

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